Implementation of an oxidative stress model for weaned piglets and effect on intestinal health

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Introduction

In modern piggery weaning is a critical period which is often associated with a reduced feed intake and serious growth check. Apart from the low zootechnical performance, the weaning period is characterized by an increased susceptibility for gastro-intestinal disorders and infections. One of the major issues being weaning diarrhea or post-weaning collibacillosis, an infection caused by enterotoxigenic E.*coli* (Lallès et al., 2004; Montagne, Pluske, & Hampson, 2003). It is clear that gastro-intestinal health is critical around weaning, as weaning is accompanied by villus atrophy (Pluske, Hampson, & Williams, 1997), reduction of the intestinal barrier function (Moeser, Ryan, Nighot, & Blikslager, 2007) and reduction of the absorption capacity (Boudry, Péron, Le Huërou-Luron, Lallès, & Sève, 2004). Oxidative stress has been proven to impair gut healt *in vivo* (Maeda et al., 2010) and *in vitro (Vergauwen et al., 2015)*; and weaning has been proven to be associated with oxidative stress (Yin et al., 2014; Zhu et al., 2013). As a consequence it can be concluded that oxidative stress might be the cause of the impaired gut health around weaning. The objective of our research was to design an oxidative stress model in weaned piglets, in which we explore the effects of paraquat as a pro-oxidative component and the possible alleviating effect of an antioxidant blend on gut oxidative status, permeability and morphology.

Materials and Methods

24 Piglets (Topics x Piétrain) from 6 litters were weaned at 19.5 (±0.5) days of age and assigned to 4 treatments. Males and females were equally distributed among the pens and every pen contained one piglet per sow. The trial included an adaptation period (d0-d27) in which all experimental animals received the same weaner diet from d0 till d21. From d22 till d27 the diet was switched to a basal starter diet. The experimental phase took place from d28 till d36-37. The control group (CON) received the same basal starter diet as before. The second pen, being the antioxidant group (AO), received the basal starter diet supplemented with an antioxidant blend containing 500mg/kg N-acetyl-L-Cystein, 100mg/kg α-tocopherol acetate, 150 mg/kg vitamin C and 0.15 mg/kg selenium. The third pen (PQ) received the basal diet supplemented with 100mg/kg paraquat dichloride. The last pen (PQ+AO) received the basal diet, plus the paraquat dichloride and the antioxidant blend. Piglets were weighed at arrival and on a daily basis during the experimental period. Feed intake per pen was measured daily during the experimental phase; average daily growth and feed conversion were recorded. Sampling was spread over 2 days (d36-d37); per day 12 piglets were slaughtered by electrical stunning followed by exsanguination. Before exsanguination a jugular venous blood sample was taken, of which the red blood cells would later be extracted for GSH/GSSG quantification. During exsanguination blood was collected in a tube containing EDTA and further processed to EDTA plasma for the determination of glutathione peroxidase (GPx) activity, glutathione transferase (GST) activity, superoxide dismutase (SOD) activity, malondialdehyde (MDA) concentration and oxygen radical absorbance capacity (ORAC). A segment of the small intestine at 75% of the total length was taken to measure the permeability of the macro-molecular marker (FITC-dextran 4kDa) in Ussing chambers. An acid extract was made of a segment at 5% and 75% of the small intestine and of a liver piece for analysis of the GSH/GSSG redox state. An analogous phosphate buffered extract was made for the analysis of GPx, SOD, GST and MDA. Finally a segment at 5% and 75% of the small intestine was taken for further histomorphological analysis. Statistical analysis was performed with SPSS 22.

Results and Discussion

There was a significant effect of treatment (p=0.008) on the GPx activity at 75% of the small intestine, with results according to our expectations. The PQ group had a significant lower GPx activity compared to the control group, and the values returned to normal in the PQ+AO group. PQ also increased the permeability for the FD4 marker at 75% of SI, but not significantly. As for the other variables there was no significant effect of treatment, nor a consistent trend in the results. It can be concluded that this trial failed to induce oxidative stress at detectable levels. This might be due to a too low concentration of paraquat added, as in literature doses administered to rats in feed range from 200 to 250 mg/kg (Ando, Igarashi, Takenaka, & Hara, 2000; Takenaka, Annaka, Kimura, Aoki, & Igarashi, 2003). In addition the adaptation period might have been too long and the lack of a fasting period prior to slaughtering might also contribute to the unexpected results.

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